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European Patent Office  
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⑪ Publication number:

0 312 858  
A1

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# EUROPEAN PATENT APPLICATION

② Application number: 88116665.6

⑤1 Int. Cl.<sup>4</sup>: C07D 233/91, C07D 249/10,  
A61K 31/415, A61K 31/41

㉚ Date of filing: 07.10.88

**Claims for the following Contracting State: ES.**

③ Priority: 22.10.87 JP 267485/87

(43) Date of publication of application:  
**26.04.89 Bulletin 89/17**

⑧ Designated Contracting States:  
BE CH DE ES FR GB IT LI

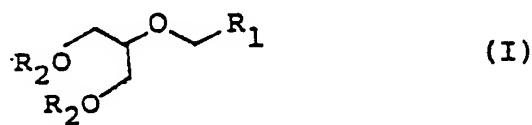
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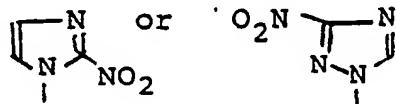
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**54 Heterocyclic derivatives, their preparation and radiosensitizing agents and antiviral agents comprising same as their active component.**

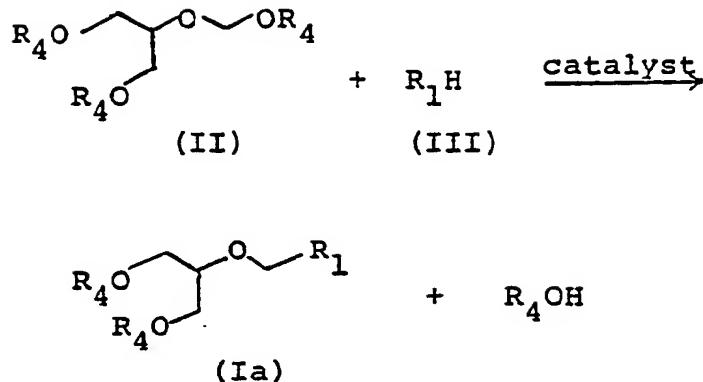
57 A novel heterocyclic derivative of formula (I):



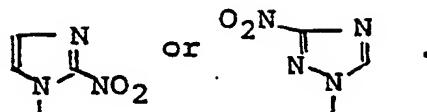
wherein  $R_1$  represents



and  $R_2$  represents hydrogen or acyl. When  $R_2$  is acyl, the derivative can be prepared, for example, by the following process:



wherein  $\text{R}_4$  is acyl and  $\text{R}_1$  is



The derivative is less toxic and has radiosensitizing activity and antiviral activity even at a low concentration. Radiosensitizing agents and antiviral agents containing the derivative as active component are also disclosed.

HETEROCYCLIC DERIVATIVES, THEIR PREPARATION AND RADIOSENSITIZING AGENTS AND ANTI VIRAL AGENTS COMPRISING SAME AS THEIR ACTIVE COMPONENT

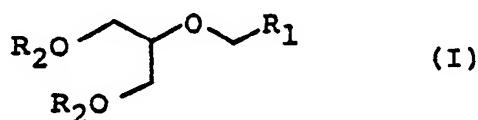
BACKGROUND OF THE INVENTION

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1) Field of the Invention:

This invention relates to a novel heterocyclic derivative of formula (I):

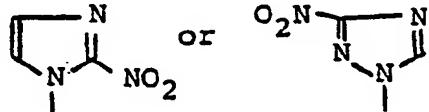
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wherein R<sub>1</sub> represents

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and R<sub>2</sub> represents a hydrogen atom or an acyl group; its preparation; and radiosensitizing agents and antiviral agents comprising the derivative as their active component.

2) Description of the Background Art:

30 Hypoxic cells in tumor tissues are strongly resistant to radiation. This fact is considered to be one of key factors that explains the obstinacy or recrudescence after radiotherapy. In view that hypoxic cells do not exist in normal tissues, it is very important to enhance the radiosensitivity of the hypoxic cells in tumor tissues in order to obtain better results from radiotherapy.

35 Meanwhile, viral infectious diseases which attack mammals including humans are contagious and bring agony and economic loss to our society. Only limited viral infectious diseases are curable by currently available antiviral agents, and new synthetic antiviral agents stand in demand.

SUMMARY OF THE INVENTION

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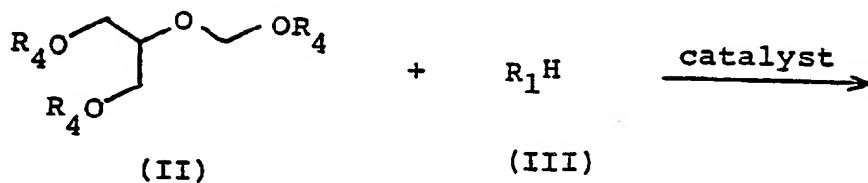
Under the above circumstances, the present inventors conducted intensive studies for developing agents capable of selectively sensitizing hypoxic cells without affecting the sensitivity of normal cells at the time of irradiation, in other words, radiosensitizing agents selectively directed to hypoxic cells (hereinafter referred to simply as radiosensitizing agents) and agents having antiviral activity. They found that 45 compounds of formula (I) have low toxicity, high radiosensitizing effect, and antiviral activity even at a low concentration. The low toxicity of the compound is notable because toxicity has long been the most serious problem in this technical field.

Accordingly, it is an object of the invention to provide a heterocyclic derivative of formula (I) and a 50 process for preparing the derivative. It is another object of the invention to provide a radiosensitizing agent and an antiviral agent comprising the derivative as their active component.

DETAILED DESCRIPTION OF THE INVENTION

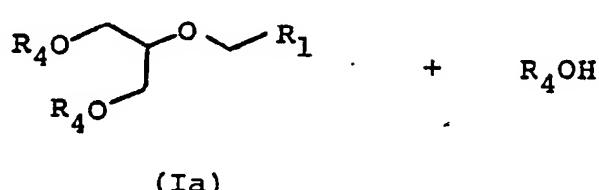
When  $R_2$  is acyl, compounds of formula (I) of this invention can be prepared, for example, by the following process:

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wherein  $R_4$  represents an acyl group and  $R_1$  has the same meaning as defined above.

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In other words, compounds (Ia) of this invention can be prepared by reacting 1,3-diacycloxy-2-acyloxymethoxypropane (II) with a compound (III). The starting compound (II) is readily obtainable according, for example, to a method described in Proc. Nat. Acad. Sci. USA, 80, 4139 (1983) by A.K. Fielol et al.

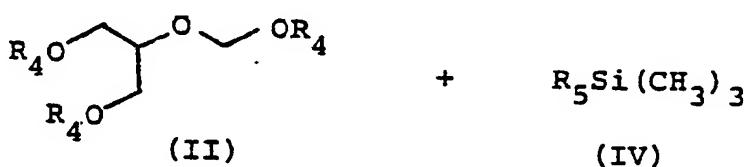
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The above reaction is carried out by melting a compound (II) and a compound (III) under a reduced pressure in the presence of a catalyst. As suitable catalyst, mention may be made of: protic acids such as *p*-toluenesulfonic acid, methanesulfonic acid and trichloroacetic acid; and Lewis acids such as anhydrous zinc chloride, anhydrous aluminum chloride and anhydrous stannic chloride. The proportion of compound (II) and compound (III) may be varied arbitrarily. Generally, it is recommended that the compound (II) be used in equivalent or a little excessive amount. The reaction temperature is preferably from 50 to 150 °C. The reaction is preferably completed in between 30 minutes to 6 hours, depending on reagent, solvent, temperature, reaction accelerator, etc.

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The compounds (Ia) of this invention can also be prepared according to the following process:

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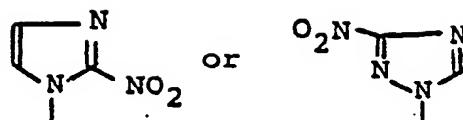
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wherein  $R_4$  is as same as defined above and  $R_5$  represents

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In other words, compounds (Ia) of this invention can be obtained by reacting 1,3-diacyloxy-2-acyloxymethoxy propane (II) with compound (IV) which is silylated derivative of compound (III).

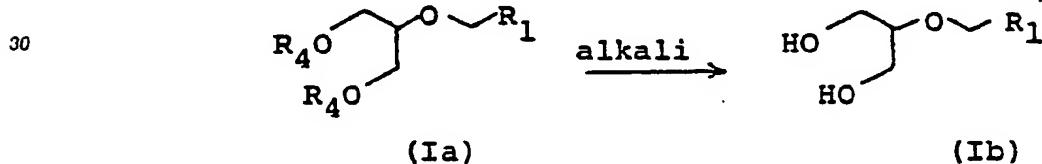
The compounds (IV) are readily obtainable by reacting their corresponding compounds (III) with excessive amounts of N,O-bis(trimethylsilyl)acetamide at room temperature or under heat while stirring. Unreacted silylation agents are removed by distillation under reduced pressure.

The reaction process according to this invention is carried out in the presence of a Lewis acid. Various Lewis acids are usable, and specific examples include anhydrous stannic chloride, anhydrous aluminum chloride, anhydrous zinc chloride, etc. They are preferably used in a catalytic amount or equivalent amount of compound (II).

The proportion of compound (II) and compound (IV) may be varied arbitrarily. In general, it is recommended that the compound (II) be used in an equimolar or a slightly excessive amount with respect to compound (IV). Various solvents can be used in this reaction, which include acetonitrile, methylene chloride, benzene, toluene, etc. The reaction proceeds at temperatures ranging from -30 to +50 °C, and generally under water cooling conditions or at room temperature. The reaction is preferably completed in between 30 minutes to 6 hours, depending on reagent, solvent, temperature, reaction accelerator, etc.

After the reaction is completed, the objective products are separated from the reaction mixture and purified according to a conventional method. For instance, the reaction mixture is subjected to extraction process, followed by condensation after washing the extract, and the residue being purified by chromatography to obtain a compound (Ia) at a high yield.

25 Going back to the general formula (I), compounds (I) having hydrogen as  $R_2$  can be prepared by deacylation of compounds (Ia) as shown below:



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One example of the deacylation process is such that proceeds in absolute alcohol containing sodium alcoholate or in absolute alcohol saturated with ammonia, at a temperature ranging from 0 °C to room temperature over a few hours to overnight. Another example of suitable deacylation is hydrolysis in water-alcohol using an organic base such as triethylamine, pyridine, etc. at a temperature ranging from room temperature to 80 °C. As suitable alcohol, lower alcohols such as methanol, ethanol and propanol may be mentioned.

Examples of the novel compounds (I) of this invention are:

45 (1) 1-[2-acetoxy-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazol,  
(2) 1-[2-acetoxy-1-(acetoxymethyl)ethoxy]methyl-3-nitro-1,2,4-triazol,  
(3) 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazol,  
(4) 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-3-nitro-1,2,4-triazol.

50 In this specification, the above compounds (1) to (4) will hereinafter be referred to as compound (1), compound (2), compound (3) and compound (4).

Compounds (I) of this invention have low toxicity as shown by the test below, and have excellent radiosensitizing ability as well as antiviral activity. They are preferably dosed 5 minutes to 5 hours prior to irradiation either orally or non-orally. They may be formed into tablets, capsules, granules, powders, sapositories or injections together with excipients, stabilizers, preservatives, modifiers, etc. as required. The administration amount depends on the patient's age, the region where tumor is produced, species and types of tumor, conditions of the patient, etc., and is preferably 0.2 to 5.0 g/m<sup>2</sup> body surface.

Action and Effect

Acute toxicity test and other tests regarding radiosensitising ability and antiviral activity were carried out using the compounds of the present invention.

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(1) Acute toxicity test

ICR strain male mice of 5 week old were intravenously or intraperitoneally administered with various  
10 compounds each dissolved in a physiological saline or in a physiological saline containing 10% DMSO. The mice were observed over 14 days and 50% death rates ( $LD_{50/14}$ ) were obtained. The results are shown in Table 1.

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Table 1

Compound Nos.	Administration	Dose (mg/kg)	Dead/Treated	LD <sub>50/14</sub>	General Status
1	intraperitoneal	720	0/2	>860	Calm down
	"	860	0/2		
2	intraperitoneal	600	0/2	790	Transient respiratory acceleration after administration, then calmed down
	"	720	0/2		
	"	860	2/2		
3	intravenous	720	0/2	>860	Calm down
		860	0/2		
4	intravenous	720	0/2	860	Calm down
	"	860	1/2		

## (2) Radiosensitivity test

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## (a) In vitro test 1

Cells used in the test: single cells of EMT-6

10 Irradiation:  $^{60}\text{Co}$  - gamma rays

Cell treatment to hypoxia:

A mixture gas of 95% nitrogen and 5% carbon dioxide was passed through cell suspension.

Survival ratio of cells:

Determined by counting colonies.

15 Radiosensitivity enhancement ratio (ER):

$$20 \quad \text{ER} = \frac{\text{Required dose for obtaining a certain biological effect in non-administered group}}{\text{Required dose in administered group for obtaining the same biological effect as obtained in non-administered group}}$$

25 The results of this test are shown in Table 2.

Table 2

30	Compound Nos.	Concentration (mM)	ER
	3	1.0	1.70
	4	1.0	1.50

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## (b) In vitro test 2

Cells used in the test: Spheroids of EMT-6

40 Irradiation:  $^{60}\text{Co}$  - gamma rays

Tested compound: Compound (3), 1mM

Determination of radiosensitivity enhancement:

45 Six particles of spheroid having a certain size were taken and placed in a culture solution containing compound (3) having a concentration of 1mM, and incubated at 37°C over 30 to 60 minutes, followed by irradiation. The spheroids were treated by trypsin and then the enhancement ratio (ER) was obtained by counting colonies.

The result obtained was:

ER of compound (3) at a concentration of 1mM = 1.55

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## (c) In vivo test

Animal: Balb/c mice

Tumor: EMT-6

55 Tested compound: compound (3), 200 mg/kg

Administration:

Compound (3) dissolved in a physiological saline was intraperitoneally administered 20 minutes prior to irradiation.

Irradiation:  $^{60}\text{Co}$  - gamma rays,  
whole body irradiation.

Determination of radiosensitivity enhancement:

Enhancement ratio (ER) was obtained from irradiation dose and reduction ratio of tumor cells.

5 The result obtained was:

ER of compound (3) (200 mg/kg) = 1.55

(3) Antiviral activity test

10 Virus: Herpes simplex virus type I  
Cells: Vero (monkey kidney cells)

Culture medium: 2% FBS MEM

A sample conditioned to contain  $2 \times 10^5$ /ml of vero cells was cultured at  $37^\circ\text{C}$  in an atmosphere of 5%  $\text{CO}_2$  for 1 day to obtain a monolayer sample. The sample was infected by HSV virus diluted with PBS (phosphate buffer). Compound (I) was dissolved in DMSO, then adjusted to have concentrations of 100 $\mu\text{g}/\text{ml}$ , 50 $\mu\text{g}/\text{ml}$ , 10 $\mu\text{g}/\text{ml}$ , 5 $\mu\text{g}/\text{ml}$  and 1 $\mu\text{g}/\text{ml}$  by 2% FBS MEM, and served as test agents. The culture cells were added with each agent separately and incubated at  $37^\circ\text{C}$  in a  $\text{CO}_2$  incubator for one day. The cytopathic effect was observed under microscope. Cells were stained by crystal violet and scored as

20 follows:

- 0: almost all cells are dead
- 1: certain effect of test agent with some dead cells
- 2: normal

The results are shown in Table 3.

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Table 3

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Compound Nos.	100 $\mu\text{g}/\text{ml}$	50 $\mu\text{g}/\text{ml}$	10 $\mu\text{g}/\text{ml}$	5 $\mu\text{g}/\text{ml}$	1 $\mu\text{g}/\text{ml}$
3	2	2	1	0	0
4	2	2	1	0	0

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Examples

This invention may be more fully understood from the following examples.

40

Example 1

1-[2-acetoxy-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazol: compound (1)

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5.6 g of 2-nitroimidazol, 12.4 g of 1,3-diacetoxy-2-acetoxymethoxypropane and 0.5 g of p-toluenesulfonic acid monohydrate were placed in a flask connected with a trap for reducing pressure by an aspirator. The flask was heated by oil bath of  $130 - 140^\circ\text{C}$  under reduced pressure while stirred. Acetic acid was distilled out as the reaction proceeded. In about 15 minutes, the reaction was completed. After cooling down to room temperature, the content was added with about 300 ml ethyl acetate and subjected to extraction. The extract was washed with saturated aqueous sodium hydrogen carbonate, and with water in this order. Then it was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by separable high performance liquid chromatography through silica gel columns using a mixture solvent (ethyl acetate - benzene) as an eluate to obtain 13.3 g of the title compound as a viscous oil material (yield: 88.6%).

55 MS(m/e): 301(M<sup>+</sup>)

IR(cm<sup>-1</sup>): 1740 (CO), 1535 (NO<sub>2</sub>), 1490 (NO<sub>2</sub>)

NMR( $\delta$ , CDCl<sub>3</sub>): 2.0 (s, 6H,  $\text{CH}_3\text{CO} \times 2$ ), 3.8 - 4.3 (m, 5H,  $-\text{CH}_2\text{OAc} \times 2$ ,  $> \text{CH}-$ ), 5.9 (s, 2H,  $-\text{OCH}_2\text{N}^+ \text{--}$ ),

7.1 (s, 1H, ring proton), 7.4 (s, 1H, ring proton)

Example 2

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1-[2-acetoxy-1-(acetoxymethyl)ethoxy]methyl-3-nitro-1,2,4-triazol: compound (2)

General procedures of Example 1 were followed to obtain the title compound as a viscous oil material

10 (yield: about 83%).

MS(m/e): 302(M<sup>+</sup>)

IR(cm<sup>-1</sup>): 1740 (CO), 1555 (NO<sub>2</sub>), 1500 (NO<sub>2</sub>),

NMR( $\delta$ , CDCl<sub>3</sub>): 2.0(s, 6H, CH<sub>3</sub>CO x 2), 3.8 - 4.3 (m, 5H, -CH<sub>2</sub>OAc x 2, >CH-), 5.9 (s, 2H, -OCH<sub>2</sub>N< ), 8.7 (s, 1H, ring proton)

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Example 3

20 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazol: compound (3)

3.01 g of 1-[2-diacetoxy-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazol (compound (1)) was dissolved in 50 ml of absolute methanol, and stirred at room temperature while being added with 5% absolute ethanol solution of sodium ethoxide dropwise until pH reached 9.0. Stirred at room temperature over 3 hours. Then 25 Dowex 50 W (H<sup>+</sup>, made by Dow Chemical) was slowly added until the liquid had a pH of 7.0. Dowex 50 W was removed by suction filtration, and the solvent was distilled off under reduced pressure. The residue was subjected to recrystallization by ethanol to obtain 2.83 g of the title compound as light yellow needles (yield: 94%).

Melting point: 88 °C

30 MS(m/e): 218 (M+1), 185, 114, 98

IR(cm<sup>-1</sup>): 3450 (OH), 1540 (NO<sub>2</sub>), 1490 (NO<sub>2</sub>)

NMR( $\delta$ , DMSO(d<sub>6</sub>)): 3.2-3.6 (m, 5H, -CH<sub>2</sub>OH x 2, >CH-), 4.6 (t, 2H, OH x 2), 5.9(s, 2H, -OCH<sub>2</sub>N< ), 7.15(s, 1H, ring proton), 7.8 (s, 1H, ring proton)

35

Example 4

1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-3-nitro-1,2,4-triazol: compound (4)

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General procedures of Example 3 were followed to obtain the title compound as colorless needles (yield: 95%).

Melting point: 132 °C

MS(m/e): 219 (M+1), 205, 185

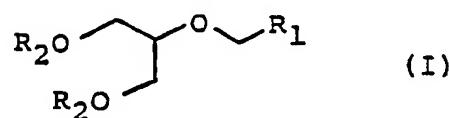
45 IR(cm<sup>-1</sup>): 3450 (OH), 1560 (NO<sub>2</sub>), 1500 (NO<sub>2</sub>)

NMR( $\delta$ , DMSO(d<sub>6</sub>)): 3.3 - 3.8 (m, 5H, -CH<sub>2</sub>OH x 2, >CH-), 4.6 (t, 2H, OH x 2), 5.8 (s, 2H, -OCH<sub>2</sub>N< ), 9.0(s, 1H, H in 5th position)

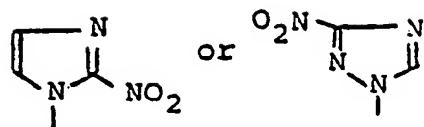
50 Claims

1. A heterocyclic derivative of the following formula (I):

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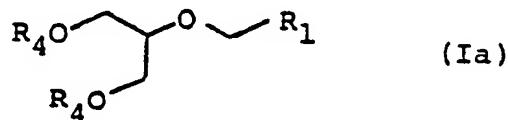


wherein R<sub>1</sub> represents

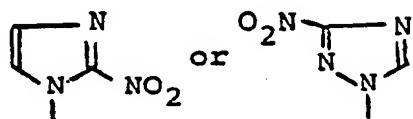


and R<sub>2</sub> represents a hydrogen atom or an acyl group.

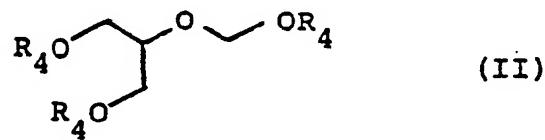
10 2. A process for preparing a heterocyclic derivative of the formula (Ia):



wherein R<sub>1</sub> represents



25 and R<sub>4</sub> represents an acyl group, which comprises reacting a compound of the formula (II):

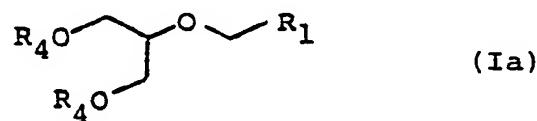


wherein R<sub>4</sub> has the same meaning as defined above, with a compound of the formula (III):

35 R<sub>1</sub>H      (III)

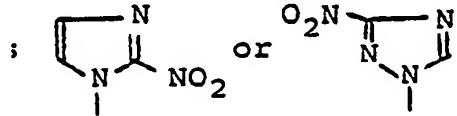
wherein R<sub>1</sub> has the same meaning as defined above.

3. A process for preparing a heterocyclic derivative of the formula (Ia):



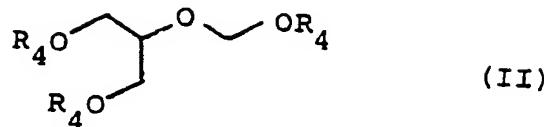
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wherein R<sub>1</sub> represents



and R<sub>4</sub> represents an acyl group, which comprises reacting a compound of the formula (III):

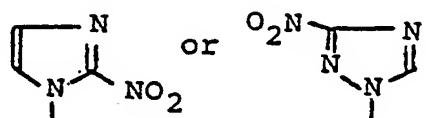
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wherein  $\text{R}_4$  has the same meaning as defined above, with a compound of the formula (IV):

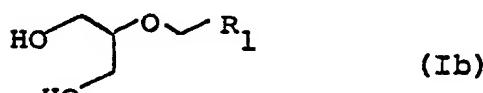


wherein  $\text{R}_5$  represents



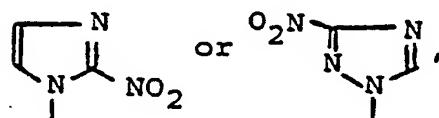
4. A process for preparing a heterocyclic derivative of the formula (Ib):

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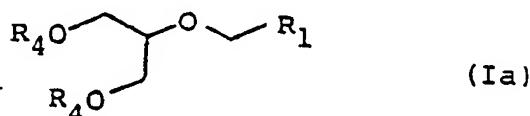
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wherein  $\text{R}_1$  represents



35 which comprises deacylating a compound of the formula (Ia):

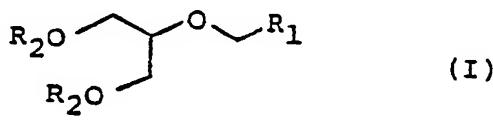
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wherein  $\text{R}_1$  has the same meaning as defined above and  $\text{R}_4$  represents an acyl group.

50 5. A radiosensitizing agent comprising as its active component a heterocyclic derivative of the formula (I):

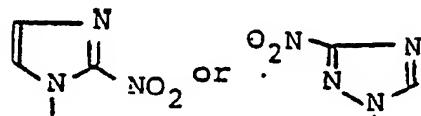
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wherein  $\text{R}_1$  represents

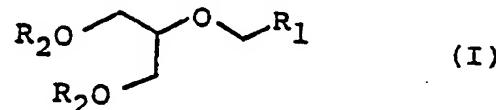
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and R<sub>2</sub> represents a hydrogen atom or an acyl group.

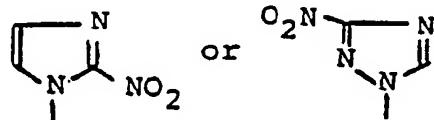
6. An antiviral agent comprising as its active component a heterocyclic derivative of the formula (I):

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10 wherein R<sub>1</sub> represents

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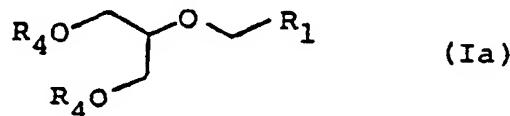


and R<sub>2</sub> represents a hydrogen atom or an acyl group.

20 Claims for the following Contracting State: ES

1. A process for preparing a heterocyclic derivative of the formula (Ia):

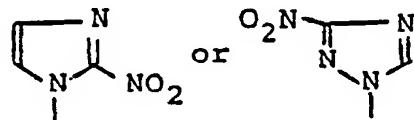
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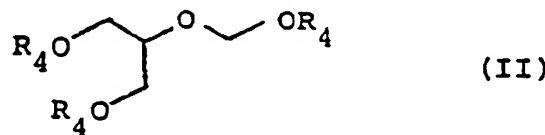
wherein R<sub>1</sub> represents

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and R<sub>4</sub> represents an acyl group, which comprises reacting a compound of the formula (II):

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wherein R<sub>4</sub> has the same meaning as defined above, with a compound of the formula (III):

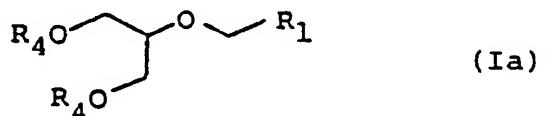


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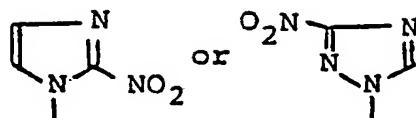
wherein R<sub>1</sub> has the same meaning as defined above.

2. A process for preparing a heterocyclic derivative of the formula (Ia):

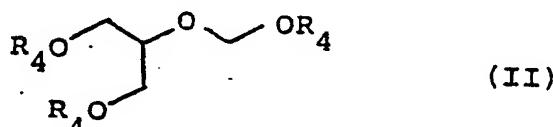
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5 wherein R<sub>1</sub> represents

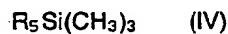


10 and R<sub>4</sub> represents an acyl group, which comprises reacting a compound of the formula (II):

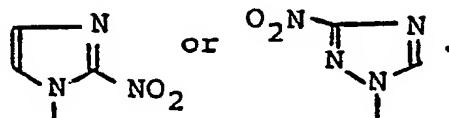


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wherein R<sub>4</sub> has the same meaning as defined above, with a compound of the formula (IV):

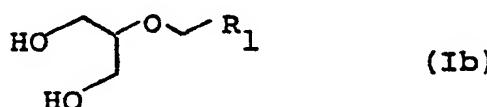


wherein R<sub>5</sub> represents



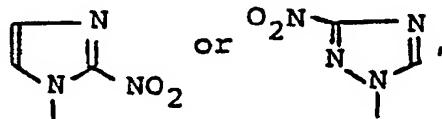
3. A process for preparing a heterocyclic derivative of the formula (Ib):

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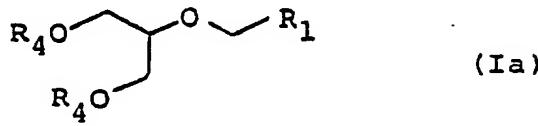
wherein R<sub>1</sub> represents



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which comprises deacylating a compound of the formula (Ia):

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wherein R<sub>1</sub> has the same meaning as defined above and R<sub>4</sub> represents an acyl group.

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European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 88 11 6665

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	WO-A-8 302 774 (RESEARCH CORPORATION) -----		C 07 D 233/91 C 07 D 249/10 A 61 K 31/415 A 61 K 31/41
A	EP-A-0 212 558 (ADEKA ARGUS CHEMICAL) -----		
A	PATENT ABSTRACTS OF JAPAN, vol. 9, no. 211 (C-300)[1934], 29th August 1985; & JP-A-60 75 430 (KAYAKU KOUSEIBUTSUSHITSU KENKYUSHO K.K.) 27-04-1985 * Abstract * -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			C 07 D 233/00 C 07 D 249/00 A 61 K 31/00
<p>The present search report has been drawn up for all claims</p>			
Place of search  THE HAGUE	Date of completion of the search  23-12-1988	Examiner  DE BUYSER I.A.F.	
CATEGORY OF CITED DOCUMENTS  X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

